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Q1
the FRS2 protein corresponding to the phosphotyrosine binding domain (PTB domain) is boxed in the figure. Putative SH2 binding regions of FRS2 are indicated in bold.

On page 32, delete the second full paragraph, and replace this paragraph with the following in accordance with 37 C.F.R. §1.121. A version with markings to show changes made is attached:

Q2
Figure 1B aligns the sequence of the PTB domain of another adaptor protein, IRS-1 (SEQ ID NO: 2), with that of FRS2 (SEQ ID NO: 3). Secondary structural elements (including α -helices and β -sheets) are boxed. Vertical lines report identical amino acids shared between the two proteins. Only two contiguous amino acids are identical within the two proteins.

On page 38 and 39, delete the last paragraph starting at line 24 on page 38 and ending on the first paragraph at line 19 on page 39, and replace this paragraph with the following in accordance with 37 C.F.R. §1.121. A version with markings to show changes made is attached:

Q3
cDNA cloning of FRS2: A pair of degenerate primers was synthesized based upon the amino acid sequence of tryptic peptide #2 (SEQ ID NO: 4) (VYENINGLSIPSASGV). PCR was performed with these two primers using cDNA prepared from mRNA isolated from 3T3 cells. The 60 base pair long product of this reaction was sequenced and found to have the correct amino acid sequence. A second round of PCR was then performed with the same cDNA using one primer chosen from the sequence of the initial 60 bp reaction product, and a second degenerate primer based upon the sequence of tryptic peptide #1 of FRS2 (SEQ ID NO: 5) (FVLGPTPVQK); this reaction gave a 170 base pair product. A third round of PCR was performed with one primer from this 170 base pair product and a T3 primer from the Bluescript vector. The 1.2 kb product of this reaction contained the sequence of peptide #1. Finally, the 1.2 kb fragment was used as a probe for screening a λ cDNA library generated

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from Swiss 3T3 cells (Stratagene). Two phage clones of λ p90-1 and λ p90-2 were isolated and further analyzed. Determination of the deduced amino acid sequences of these two clones revealed a long open reading frame (ORF) that contained the sequences of the four tryptic peptides that were isolated from purified FRS2.

On page 44 and 45, delete the last paragraph starting at line 24 on page 44 and ending on the first paragraph at line 12 on page 45, and replace this paragraph with the following in accordance with 37 C.F.R. §1.121. A version with markings to show changes made is attached:

EXAMPLE 4: cDNA CLONING OF FRS2

Q4
Peptide sequences from FRS2 were used to design oligonucleotides for amplification by polymerase chain reaction (PCR) of cDNA prepared from mRNA isolated from NIH-3T3 cells. A 1.2 kb PCR product contained the sequences of proteolytic peptide #1 (FVLGPTPVQK) (SEQ ID NO: 5) and peptide #2 (VYENINGLSIPSASGV) (SEQ ID NO: 4) from FRS2. The 1.2 kb PCR product was then used as a probe for screening a cDNA library from NIH-3T3 cells. Two overlapping clones, λ p90-1 and λ p90-2, were isolated. Determination of the nucleotide sequences of the two clones demonstrated that λ p90-2 contained the sequences of all four tryptic peptides isolated from FRS2 and that λ p90-2 represents a full-length cDNA clone of FRS2.

On page 45 and 46, delete the last paragraph starting at line 13 on page 45 and ending on the first paragraph at line 4 on page 46, and replace this paragraph with the following in accordance with 37 C.F.R. §1.121. A version with markings to show changes made is attached:

Q5
The deduced amino acid sequence of FRS2 determined from clone λ p90-2 is presented in Figure 1A. The coding sequence of FRS2 begins at nucleotide number 308. The first methionine is within a Kozak consensus sequence, and is followed by an open reading

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frame (ORF) of 1527 base pairs, ending with a stop codon at nucleotide 1834. This ORF encodes a protein containing 508 amino acids with a predicated molecular mass of 56,800 daltons. The sequence of FRS2 contains a consensus myristylation sequence (MGXXXS/T) at the amino terminus of the molecule MGSCCS (SEQ ID NO: 6). Resh, 1994, Cell 76:411-413. In addition, FRS2 contains a stretch of 120 amino acids (residues 11 to 139) with 29% sequence identity to the PTB (phosphotyrosine binding) domain of IRS1 (Figure 1B). Sun et al., 1991, Nature 352:7377. It has been shown that the PTB domain of IRS1 binds to a tyrosine phosphorylated NPXYp sequence in the juxtamembrane region of the insulin receptor. White et al., 1988, Cell 54:641-649. FRS2 also contains two potential Grb2 binding sites (NYEN (SEQ ID NO: 7) and NYVN (SEQ ID NO: 8)) (Figure 1B).
